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14. ABSTRACT
The general objective of this study was to identify the conditions under which electrophysiological methods could be used to investigate hearing in pinnipeds and to establish how such measurements compared with those obtained using standard behavioral techniques. To meet this objective, a research partnership was established involving participants from academia (University of California Santa Cruz), government (Navy Marine Mammal Program), and private industry (BIOMIMETICA); this partnership was later expanded to include a foreign specialist from the Russian Academy of Sciences. During this effort, data collection systems and research protocols were adapted and field tested for use with pinnipeds. Individuals representing three pinniped species (California sea lions, harbor seals, and northern elephant seals) were tested, either voluntarily or while under anesthesia. Research accomplishments included measuring and characterizing auditory brainstem responses, development of response amplitude maps, and completion of a series of audiometric experiments.

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FINAL REPORT

GRANT #: N00014-56-J-0011

PRINCIPAL INVESTIGATOR: Colleen Reichmuth Kastak, Ph.D.

INSTITUTION: University of California Santa Cruz

OBJECTIVE: To identify the conditions under which auditory evoked potentials can be used to investigate hearing in three pinniped species.

APPROACH: The approach was to cooperate with NOPP partners from government (J. Finneran and P. Moore from the Navy Marine Mammal Research Program) and private industry (D. Houser from BIOMIMETICA) to develop the technology and testing protocols required to successfully obtain neuro-physiological audiometric data from pinnipeds. The subjects were California sea lions (Zalophus californianus), harbor seals (Phoca vitulina), and northern elephant seals (Mirounga angustirostris) that were either trained for voluntary participation in research or were tested while anesthetized for rehabilitation purposes. Auditory evoked potentials were elicited by brief acoustic stimulation and characterized for each species. Monopolar recording techniques were used to determine the species-specific electrode configurations that yield optimal response amplitudes. Using these configurations, stimulus parameters including signal type, bandwidth, presentation rate, and amplitude were manipulated in order to evaluate different aspects of auditory physiology, including hearing sensitivity.

ACCOMPLISHMENTS: With the assistance of our collaborators, two independent systems for the collection and analysis of auditory evoked potentials were developed and tested. Working with both trained animals and wild animals undergoing rehabilitation, progress was made in three general areas: 1) response characterization was conducted for each of three species, 2) species-specific optimal electrode configurations were determined, and 3) a series of audiometric experiments were completed. These data were analyzed and compared to findings from behavioral experiments. The results obtained provide a general understanding of species-typical auditory evoked responses, which is informing current efforts to investigate various aspects of auditory function in pinnipeds.

1) In order to identify the basic characteristics of the auditory brainstem response (shape, latency, and amplitude), auditory evoked potentials were obtained for the three species under investigation in response to brief, broadband clicks. The maximum averaged response amplitudes obtained for the California sea lions and harbor seals tested were <1.5 μV. This amplitude is comparable to that obtained for human subjects, but is substantially smaller (about an order of magnitude smaller) than those reported for dolphins and porpoises. The northern elephant seals tested exhibited a much smaller auditory response, about 200-300 nV (about a third of the size of the other pinnipeds).

2) To determine what type of electrodes and what electrode positions should be used in studies of auditory evoked potentials in pinnipeds, two California sea lions, two northern elephant seals, and one harbor

seal were opportunistically tested while undergoing routine anesthesia at a rehabilitation facility (The Marine Mammal Center, in Sausalito, California). Response amplitude maps were generated for each individual by varying the position of the active electrode along the sagital and transverse planes of the head. The resultant maps establish the optimal electrode position determined for each of the three species tested. Two types of electrodes were used: small, subdermal stainless steel or platinum needle electrodes that could be inserted ~1 cm into the skin, and gold plated discs that could be placed on the shaved surface of the skin. Both electrode types were shown to be effective at collecting the neural responses of interest, however, the needle electrodes provided significantly higher amplitude response amplitudes than the surface electrodes.

3) An additional series of experiments was conducted to characterize the auditory evoked responses of the subjects as a function of changing stimulus parameters. These included: a) progressively decreasing stimulus level, b) progressively increasing stimulus presentation rates, c) progressively narrowing the stimulus bandwidth, and d) altering the stimulus type. During the course of these experiments, repeated testing with modification allowed us to identify and reduce stimulus artifacts and to measure and improve signal-to-noise ratios in our records. We determined that stimulus presentation rates up to several dozen per second did not result in decreased response amplitude. By combining progressive decreases in stimulus amplitude with progressive narrowing of the stimulus bandwidth, we were able to generate frequency-specific hearing curves using auditory brainstem responses that could be compared to those obtained using standard behavioral methods. Finally, by testing a range of stimulus types including broadband clicks, click trains, band-narrowed clicks, tone pips, and sinusoidally amplitude modulated (SAM) stimuli, we evaluated the benefits and limitations of using electrophysiological methods to obtain rapid information about auditory sensitivity and auditory function.

This research effort was aided by additional support provided by a Science and Technology Engagement Program (STEP) award from ONR Global to C. Reichmuth Kastak. This award expanded the research partnership to include Alexander Supin of the Severtsov Institute of Ecology and Evolution, at the Russian Academy of Sciences. The participation of Dr. Supin in this project served to improve and expand research efforts as well as address the overarching goal of methodological standardization amongst researchers using electrophysiology to measure hearing in marine mammals.

CONCLUSIONS: Results show that electrophysiological techniques can be applied to obtain rapid audiometric information from pinnipeds. Testing parameters, however, are species-specific and appropriate protocols must be used. The evoked potential technique appears especially well suited for studying certain aspects of auditory function such as temporal processing using supra-threshold level stimuli. However, use of the technique for examining frequency-specific hearing thresholds is limited at lower frequencies. Additionally, it appears unlikely that SAM stimuli, which are used to obtain a high degree of frequency specificity in other marine mammals, will prove to be appropriate for testing the full hearing range of pinnipeds unless technology development allows for enhanced extraction of low-amplitude signals

from noise. Electrophysiological methods will likely be most useful for investigating auditory function, mid- to high-frequency hearing, and for gross diagnostic assessment in rehabilitation settings.

SIGNIFICANCE: The work accomplished in this research partnership is invaluable to ongoing and future efforts to develop and apply standardized electrophysiological methods for marine mammal auditory assessment. The findings establish a solid foundation for continuing efforts to use evoked potential measurements to study the hearing of pinnipeds. This effort establishes that electrophysiological techniques can be successfully used to obtain data on auditory function and sensitivity with pinnipeds, and although there are specific limitations that must be considered when this method is applied, it is a promising tool for future research and conservation efforts.

PATENT INFORMATION: Not applicable.

AWARD INFORMATION: Not applicable.

PUBLICATIONS AND ABSTRACTS:

Reichmuth Kastak, C., Kastak, D., Finneran, J.J., Houser, D.S., and Supin, A. (2005) Electrophysiological methods for hearing assessment in pinnipeds. Journal of the Acoustical Society of America, 117:2408.

Mulsow, J., Reichmuth Kastak, C., and Supin, A. Ya. Measurement of auditory temporal resolution in the California sea lion (*Zalophus californianus*) using evoked potentials. Proceedings of the 16th Biennial Conference of the Biology of Marine Mammals, 2005 Dec 12-16, p. 201.

Reichmuth Kastak, C., Supin, A. Ya., Mulsow, J. (in preparation) Characterization of auditory evoked responses in pinnipeds. To be submitted to: Aquatic Mammals: special issue on Electrophysiological Measurements of Hearing in Marine Mammals.

Mulsow, J., and Reichmuth Kastak, C. (in preparation) An evoked potential study of temporal resolution in pinnipeds. To be submitted to: Aquatic Mammals: special issue on Electrophysiological Measurements of Hearing in Marine Mammals.